1. Standardize Parts

1.1 PCR amplify 25aa and 38aa (50ul volume)

2013/8/2

TaKaRa LA Taq	0.5ul
dNTP	3ul
Template	1ul
Primer f	1ul
Primer r	1ul
10×buffer	5ul
ddH2O	38.5ul
Total	50ul

25aa tm=67.9℃

38aa tm=61.6℃

95℃	2min	
95℃	30S	
tm	30S	30cycle
72℃	1min40s	
72℃	5min	
4℃	∞	

Lip temperature 105℃

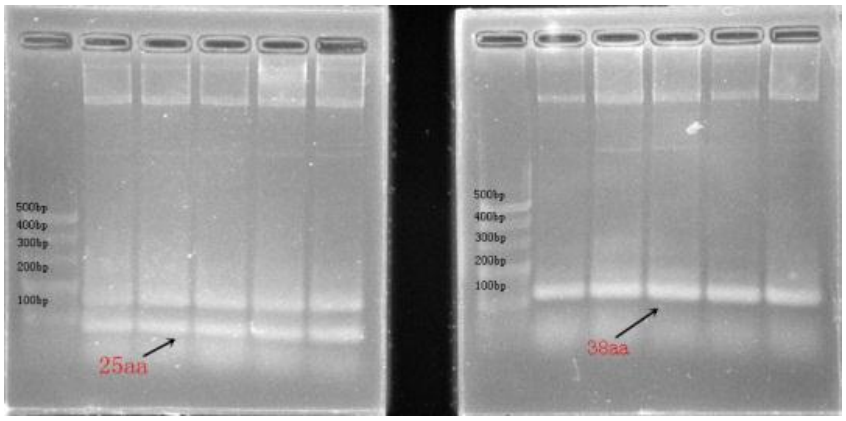

Fig. 1. 8.02 38AA 25AA PCR

Gel Extraction(AxyPrep DNA Gel Extraction Kit)

1.2 PCR products double enzyme cutting

2013/8/9

EcoRI	1ul
SpeI	1ul
H buffer	3ul
DNA	20ul
ddH2O up to	30ul

1.3 PSB1C3 double enzyme cutting

2013/8/9

EcoRI	1ul
SpeI	1ul

H buffer 3ul

DNA 20ul

ddH2O up to 30ul

1.4 Ligation

2013/8/9

25aa/38aa 6ul

PSB1C3 2ul

T4 DNA ligase 1ul

Buffer 1ul

ddH20 up to 10ul

1.5 Electroporation

2013/8/10

Add 1ul ligation products to compement cells

1.6 Validation

2013/8/13

Extract Plasmid DNA by using Plasmid DNA Extract kit

Digest Plasmid DNA by EcoRI PstI

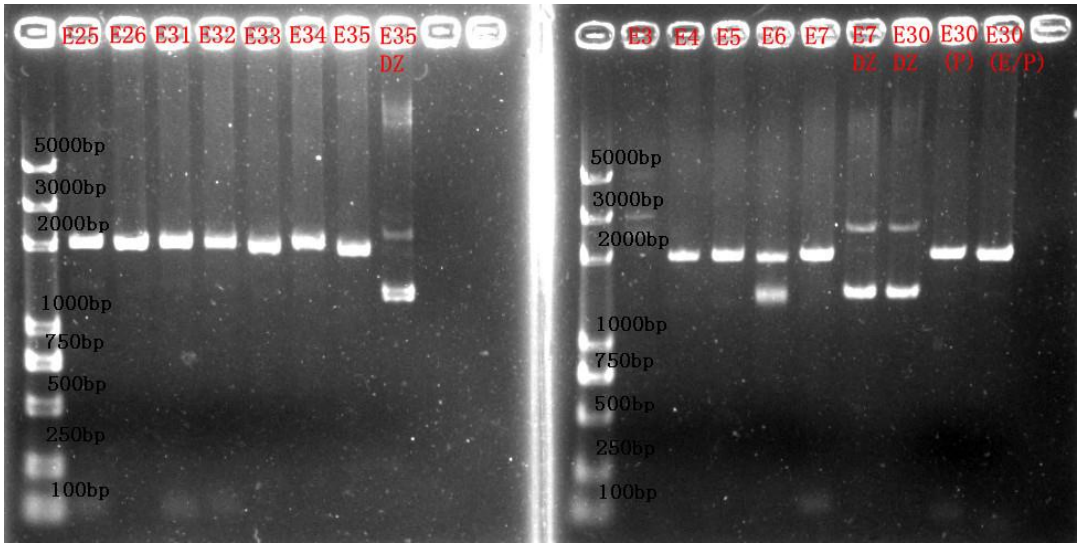

Fig. 2. 8.13 38 25 psb1c3 ml jd

Choose E7 E25 E30 E31 to sequence

E7 E25 E31 was succeed

2. Assembly standard part

2.1 Add 1-1B behind 25aa

2.1.1 25aa digest by EcoRI SpeI

2013/8/9

EcoRI 1ul

SpeI 1ul

H buffer 3ul

DNA 20ul

ddH2O up to 30ul

2.1.2 1-1B digest by EcoRI XbaI

2013/8/9

DNA	20ul
EcoRI	1ul
XbaI	1ul
M Buffer	3ul
BSA	3ul
ddH2O up to	30ul

2.1.3 Ligation

2013/8/9

25aa(E,S)	6ul
1-1B(E ,X)	2ul
T4 DNA ligase	1ul
Buffer	1ul
ddH20 up to	10ul

2.1.4 Electroporation

2013/8/10

Add 1ul ligation products to compement cells

2.1.5 Validation

2013/8/13

Extract Plasmid DNA by using Plasmid DNA Extract kit

Digest Plasmid DNA by EcoRI PstI

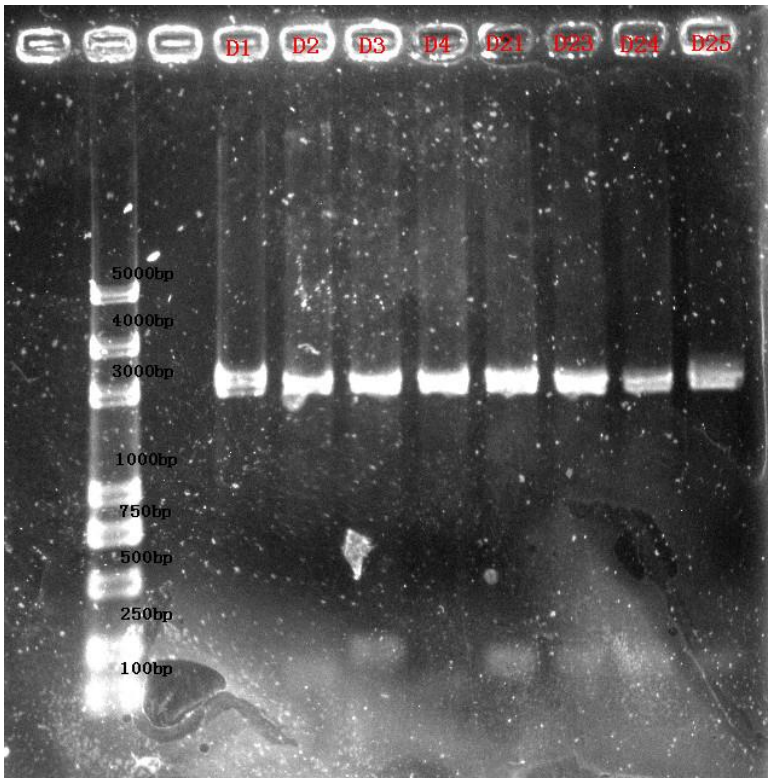

Fig. 3. 8.13 38 25 1-1b ml jd

Choose D2 D3 D21 D24 to sequence

D24 was succeed

2.2 Add 1-11E before 38aa

2.2.1 38aa digest by XbaI PstI

2013/8/19

DNA	20ul
XbaI	1ul
PstI	1ul
Buffer M	3ul
BSA	3ul
ddH2O up to	30ul

2.1.2 1-11E digest by SpeI PstI

2013/8/19

DNA	20ul
SpeI	1ul
PstI	1ul
Buffer H	3ul
ddH2O up to	30ul

2.1.3 Ligation

2013/8/19

38aa(X,P)	6ul
1-11E(S ,P)	2ul
T4 DNA ligase	1ul
Buffer	1ul
ddH20 up to	10ul

2.1.4 Electroporation

2013/8/19

Add 1ul ligation products to compement cells

2.1.5 Validation

2013/8/20

Extract Plasmid DNA by using Plasmid DNA Extract kit

Digest Plasmid DNA by EcoRI PstI

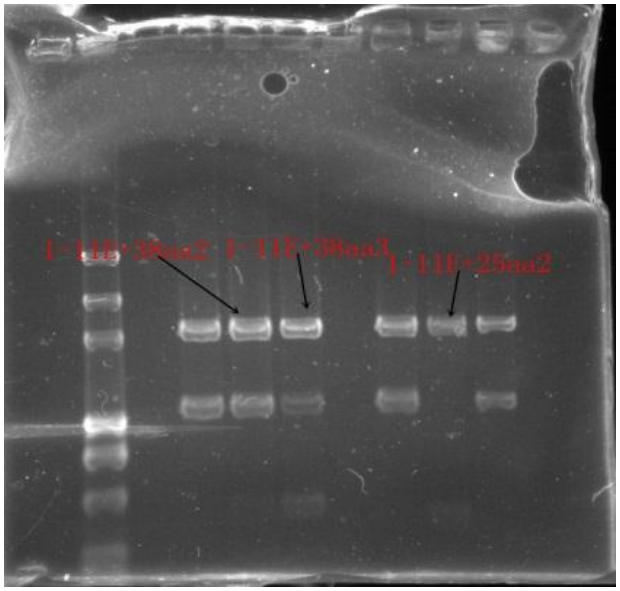

Fig. 4. 8 20 1-11E+E7,1-11E+D24-SMQ

Choose 1-11E+38aa2 1-11E+38aa3 1-11E+25aa2 to sequence
1-11E+38aa2 was succeed

3. Ligate 25aa/38aa to PHT304

3.1 PHT304 digest by EcoRI PstI and gel extraction

2013/8/29

DNA	16ul
EcoRI	1ul
PstI	1ul
10×H Buffer	2ul
ddH2O up to	20ul

Gel Extraction(AxyPrep DNA Gel Extraction Kit)

3.2 25aa/38aa with 1-11E and 1-1B digest by EcoRI PstI

2013/8/31

DNA	16ul
EcoRI	1ul
PstI	1ul
10×H Buffer	2ul
ddH2O up to	20ul

3.3 Ligation

2013/8/31

38aa/25aa	10ul
Pht304	7ul
T4 DNA ligase	1ul
Buffer	2ul
ddH20 up to	20ul

3.4 Electroporation

2013/9/11

Add 2ul ligation products to compement cells

2013/9/15

3.5 Validation

2013/9/12

Extract Plasmid DNA by using Plasmid DNA Extract kit

Digest Plasmid DNA by EcoRI PstI

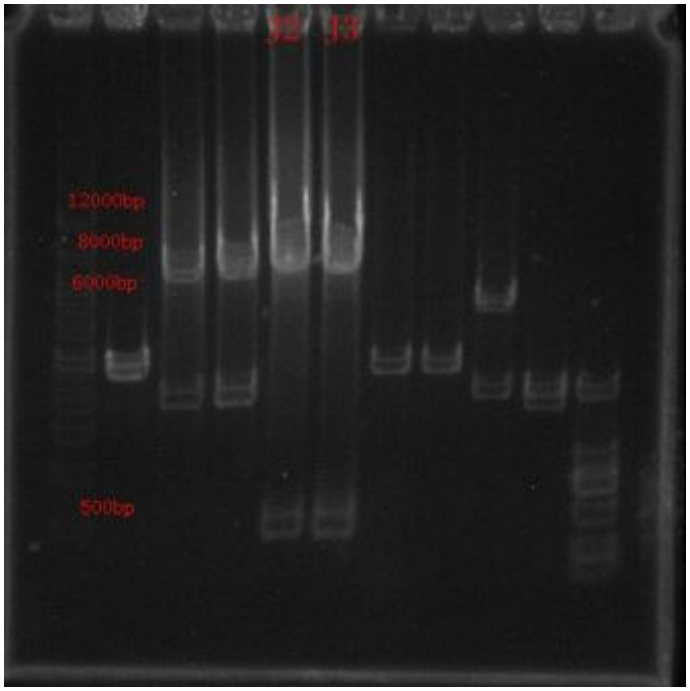

Fig. 5. J2 J3 digest by EcoRI PstI

Choose J2 J3 to sequence

J2 J3 was succeed

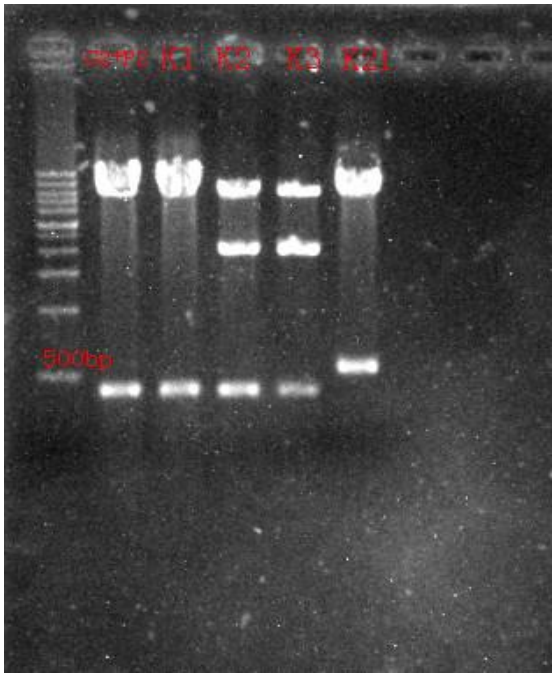

Fig. 6. G2+P2 K1-K3 K21 digest by EcoRI PstI

Choose K1 K21 to sequence

K1 K21 was succeed

PS: J2 and J3:38aa+PHT304

K1:25aa+PHT304

K21:38aa+25aa+PHT304

4. Electroporation 25aa and 38aa with PHT304 to BS

The Plasmid DNA were transformed into *Bacillus Subtilis* competent cells(Add 2ul ligation to compement cells), coated plates, select monoclonal colony PCR.

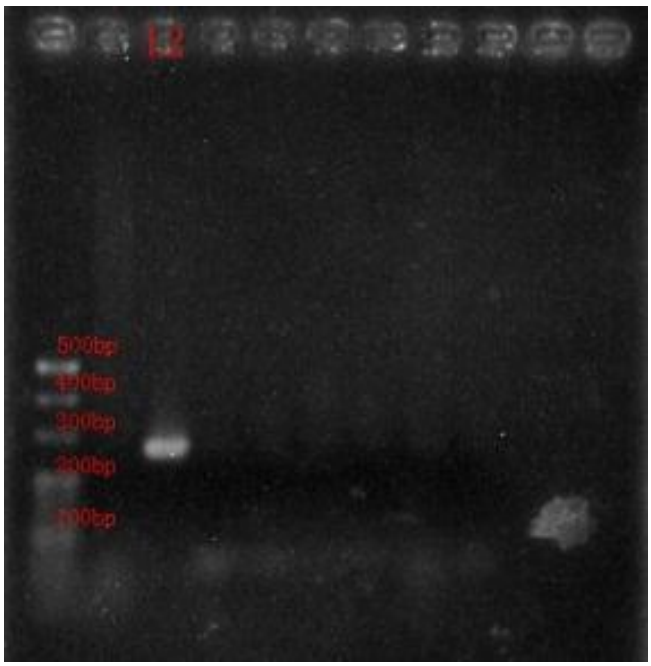

Fig. 7. 9.21 PCR ROPA L1-L4 L21-L24

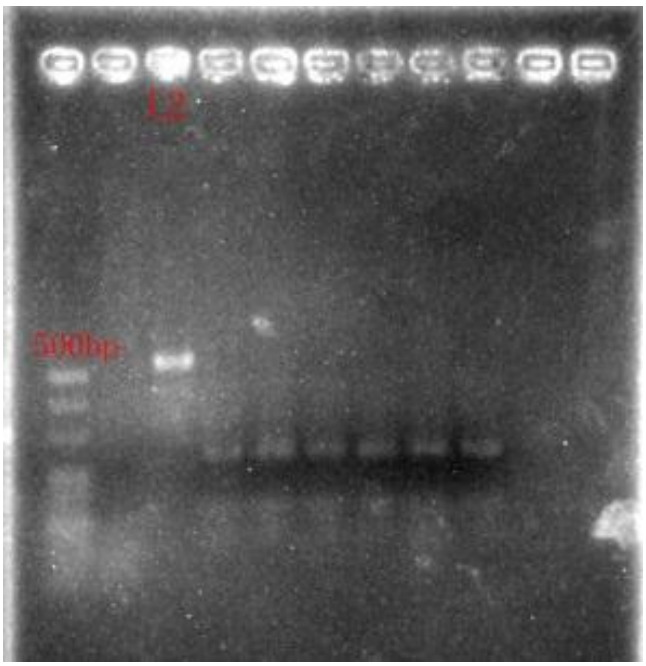

Fig. 8. 9.21 PCR M13 L1-L4 L21-L24

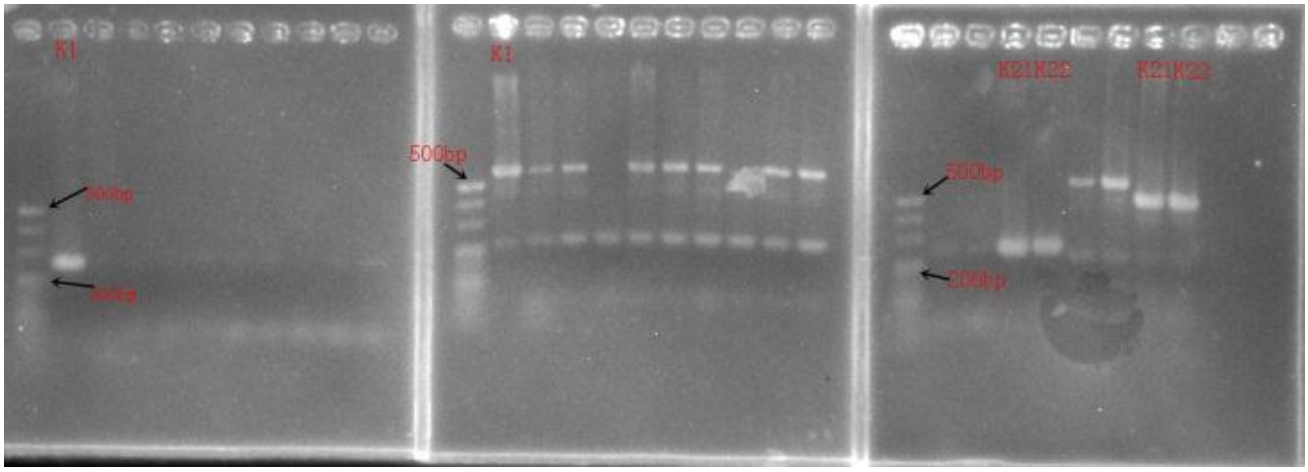

Fig. 9. 9.24 PCR ropa M1-M10 m13fr M1-M10 ropa M11-M12 M21-M22 m13fr M11-M12 M21-M22

Choose L2-1 L2-2 K1 K21 K22 to sequence

L2-1 L2-2 K1 K21 K22 was succeed

5. Antibacterial peptide activity determination

＋： **positive control**

－： **negative control**